IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	)	Group Art Unit: 1647
COX	)	Examiner: Stoica, Elly Gerald
Serial No.: 10/773,654	)	Confirmation No.: 7639
Filed: February 5, 2004	)	DECLARATION OF GEORGE COX
Atty. File No.: 4152-1-PUS-6	)	(under 37 CFR § 1.132)
For: "CYSTEINE VARIANTS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR"	)	***Via Electronic Filing***

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, George Cox, declare as follows:

1. I the inventor of the above-referenced patent application and am familiar with the application. I am a skilled artisan in the fields of molecular and cellular biology and have been involved with the experiments described in paragraphs 4-5 below.

2. This Declaration is being submitted in conjunction with an Amendment and Response to an Office Action having a mailing date of August 3, 2006.

3. The following discussion is provided in response to the Examiner's rejections of Claims 24-46 under 35 U.S.C. § 112, first paragraph. Specifically, the data presented in the following paragraphs demonstrate that the present inventor and colleagues have constructed two cysteine muteins of granulocyte-macrophage colony-stimulating factor (GM-CSF) that fall within the scope of the present claims, and have shown that the muteins are biologically active in an *in vitro* cell-based proliferation assay for GM-CSF activity. Moreover, the following data demonstrate that modification of the free cysteine moieties on one of the muteins with polyethylene glycol (PEG) does not inhibit the biological activity of the muteins.

4. Using the methods and techniques described in Examples 1 and 7 of the above-referenced application, muteins containing a single cysteine insertion were constructed in the human GM-CSF gene and were expressed in an *E. coli* expression system. The reference to position numbers is made with regard to SEQ ID NO:8 of the specification, which represents the amino acid sequence of the mature GM-CSF protein (see Example 7). The resulting muteins include insertions preceding the first amino acid of the mature protein (referred to as *-1C) and following the last amino acid of the mature protein (referred to as *-128C).

5. The muteins described in paragraph 4 above were expressed in *E. coli* as periplasmically secreted proteins using the *E. coli* STII signal sequence and tested for biological activity *vs.* a wild type GM-CSF control (obtained from R&D Systems, Inc., Minneapolis, MN) in an *in vitro* cell-line based proliferation assay. The two GM-CSF cysteine muteins (*-1C and *128C) described in paragraph 4 were purified to homogeneity. The *-1C mutein was also modified with polyethylene glycol ("PEGylated") using techniques described in Example 1 of the above-identified application. The PEGylated form of the *-1C cysteine mutein was purified away from any unmodified material. The purified cysteine muteins and the purified PEGylated cysteine mutein were assayed for biological activity *vs.* a wild type GM-CSF in an *in vitro* cell-line proliferation assay using the human TF-1 cell line. Both of the purified cysteine muteins and the purified PEGylated cysteine mutein were biologically active. The EC_{50} of the purified cysteine muteins and the purified PEGylated cysteine mutein ranged from indistinguishable from the EC_{50} of the wild type GM-CSF control to within approximately 2-fold of the EC_{50} of the wild type control.

6. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date: November 3, 2006 By: George Cox

George Cox